ECOLOGY

Prevalence of critically endangered European eel (Anguilla anguilla) in Hong Kong supermarkets

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European eel (Anguilla anguilla) is a critically endangered species requiring CITES permits for international trade. Despite the fact that no imports to Hong Kong were declared within the last 2 years, our study found that this species is still commonly sold in major supermarket chains across Hong Kong. In a COI barcoding survey of 49 retail vendors encompassing 13 brands, 9 of 13 carried A. anguilla, and 45% of all eel products available at retail outlets (n = 49) were unambiguously identified as A. anguilla. Considering the visual similarity of eel species and disproportionate amount of undeclared A. anguilla available for consumption, this finding raises urgent concerns regarding the enforcement of international CITES trade regulations. Furthermore, the prevalence of A. anguilla in supermarkets highlights how illicit wildlife products are not solely limited to specialized affluent buyers; some species have entered mainstream distribution networks for the average consumer.

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INTRODUCTION

Species and current conservation status

One of 16 anguillid eel species (1), the European eel (Anguilla anguilla) is a catadromous and panmictic freshwater eel whose continental nonbreeding range is distributed across Europe, North Africa, and Mediterranean Asia (2). The species is vulnerable to various potential threats at multiple developmental stages of its complex life history, which include habitat loss and modification, migration barriers, pollution, parasitism, fluctuating oceanic conditions, as well as exploitation (3–9). A. anguilla is commercially harvested across all continental stages of its life cycle, and juvenile glass eels are wild caught to "seed" farming operations in Europe and East Asia (to date, captive breeding has not been economically viable) (10).

While the exact contribution of anthropogenic impacts and changes in ocean-atmospheric conditions affecting the northern hemisphere eel species remains unclear, a combination of factors has resulted in declines in recruitment of Anguilla japonica (the Japanese eel), causing East Asian farms to seek alternative sources beyond species of local provenance (9, 11). Along with other combined pressures, this has resulted in unsustainable exploitation of A. anguilla with recruitment declines of 90% since the 1980s (12). In 2007, European eel was listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) (10, 13-15). While not immediately threatened with extinction, Appendix II-listed species may become so without controls on international trade. Hence, import and export permits are required when Appendix II-listed species are moved across international borders. On the basis of not being possible to perform a nondetriment finding, a zero-import/export policy was imposed and all commercial trade of A. anguilla to and from the European Union was banned from 3 December 2010 onward (15). A. anguilla is currently assessed as "critically endangered" by the International Union for Conservation of Nature (IUCN) Red List (16), and illegal trade of A. anguilla is recognized as one of the most serious wildlife crime problems the European Union faces (17, 18). Europol estimates that

100 MT (equal to approximately 300 million eels) were trafficked from Europe to Asia in the 2017/2018 glass eel catch season (19).

International trade of European eel

Eel farming makes up 90% of eel production and relies heavily on wild-caught glass eels (10). Glass eels are a highly lucrative commodity; for example, 1 kg of European eel fry on the black market can contain up to almost 3500 individuals, sometimes fetching as high as EUR 6250 per kilogram in East Asia (18). While growth rates and market prices for species vary, the increase in biomass over a 2-year growth period has been found to be roughly $1000\times$ and corresponds to a large return on investment (10, 18). The high-value trading associated with eel farming has inevitably attracted attention from criminal organizations, with reports of eel trafficking closely linked to criminal syndicates worldwide (2, 19, 20).

The European Union ban on European eel exports has preceded a shift in European eel exports coming from North African countries and also resulted in increased exploitation of other anguillid species in North America and the Indo-Pacific (13). Before the ban, 93 to 98% of live eels exported from North Africa were destined for European countries; following the ban, 91 to 93% of exports are now bound for East Asia (13). Although North African countries have their own policies regulating anguillid trade, there is evidence that the European Union ban can be circumvented by states within the range of the European eel but not subject to the European Union's regulations to access European eel populations (13). Regardless, all European eel, whether originating from North Africa or Europe, would require the proper CITES permits and documentation when traded internationally (10, 13, 21). Despite regulations and increasing seizures in recent years, the illicit eel trade continues to pose challenges for enforcement (10, 21). Demand for eel is mostly from East Asia, and the Hong Kong Special Administrative Region is an important hub for regional glass eel trade through which Europe-Asia trafficking routes have been documented (10, 21–23). Illegally traded species can often evade customs inspection due to visual similarity between legal and illegal species, which present even greater challenges in identifying processed products (2). In both live and processed forms, European eel fry are extremely difficult to visually distinguish from other anguillids such as A. japonica and Anguilla rostrata (2), and DNA-based identification using molecular methods is required

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for prosecution in court (2). While *A. japonica* and *A. rostrata* are considered "endangered" by the IUCN Red List (24, 25), only *A. anguilla* is classified as critically endangered and listed in CITES Appendix II. As a result, *A. anguilla* can be laundered with legal products and transported across borders undetected unless identified by genetic tests, which—despite recent developments of improved rapid testing protocols (26)—are still limited by time and cost.

Data downloaded from the CITES trade database website (https://trade.cites.org, accessed 29 March 2019) show there were no declared instances of legal European eel meat importation into Hong Kong in 2017 or 2018. As there are no eel aquaculture facilities in Hong Kong, all live or processed eel products would have been imported from abroad. Although CITES permits are required, the Chinese government often does not report trade between Hong Kong and China to CITES, potentially obscuring the path of traded eels (2). The CITES Animal Committee identified issues with regard to countries reporting eel exports and imports to the CITES trade database and recommended amendments for the description of specimen codes to increase future reporting procedures and data quality (27). The CITES Conference of the Parties 18 (CoP18) further adopted Decision 63 that encourages eel range states (A. anguilla and other Anguilla species) to develop and implement measures to improve the traceability of eels in trade (28). While discrepancies between European, North African, and Asian CITES and customs trade data certainly exist (2), we focused our analysis on CITES trade reports as the Protection of Endangered Species of Animals and Plants Ordinance (Cap. 586, which governs the permit requirements of import/exports into and out of Hong Kong) is based on species listings in CITES Appendices (2). In addition, CITES trade data identify to the species level, while other databases such as United Nations Comtrade are not as specific, and, in some instances, Anguilla species are aggregated with other fish species such as carp and catfish (e.g., Harmonized Commodity Description and Coding System code 030439, which encompasses eight different genera). On the basis of the official CITES trade reports, it is expected that European eel products found in Hong Kong should be few to none.

Retail outlets of European eel in Hong Kong

In Hong Kong, raw or live eel is often purchased from wet markets, while processed or packaged products are commonly purchased from supermarkets. Ninety-five percent of Hong Kong's food consumption is dependent on imported products, with almost 60% of the population relying on supermarkets for their day-to-day shopping compared with an estimated 30% for traditional wet markets (29). Supermarkets and convenience stores in Hong Kong are a virtual duopoly, with almost all retail outlets owned by one of two major multinational retail groups. Thus, any one retail group's choice of supplier has a large impact on what is imported and made readily available to the general population. The two multinational corporations that control the Hong Kong supermarket sector both have a large worldwide presence, spanning Southeast Asia, East Asia, Russia, and Europe. As a central node in pan-Asian shipments, Hong Kong is both a key point of entry for shipments into China and a massive exporter of Chinese goods.

RESULTS

Genetic survey of eel products in supermarkets

All extracted DNA successfully amplified, yielding a clear single band at the target length when visualized on a 1.5% agarose gel. A total of

22 of 49 eel products were genetically identified as *A. anguilla* using *COI* barcoding. Of the retail brands surveyed in the targeted study, 9 of 13 vendors were found to sell European eel packaged as various food products and labeled only as "eel." Each retail outlet was given a letter (A or B) denoting retail group, followed by a number indicating the associated market brand under that conglomerate. While animal product species names are neither compulsory nor common in Hong Kong, in one instance, live eel was labeled as *A. japonica*, but DNA results identified the specimen as *A. anguilla*.

For all samples, there was no major disagreement among the identification methods, i.e., GenBank top hit, Barcode of Life Identification (BOLD ID), and our tree analysis all resulted in the same species ID with identity matches greater than 99% (see table S1).

In addition, misidentified accessions in open source sequence databases create a potential issue for Basic Local Alignment Search Tool (BLAST)–based identification. The top dissimilar hit in each BLAST results table was checked against BOLD for verification. Several GenBank accessions did not match their BOLD IDs and so were discarded.

GenBank accessions KJ564271, KJ564271, and KT951835 are listed as *A. rostrata*, *A. rostrata*, and *A. japonica*, respectively; however, when searched against BOLD, they all return as *A. anguilla* with a 100% match. Because of this discrepancy, these accessions were ignored. Accessions KJ948424 and EU266379 were also ignored, as they were both labeled as *A. japonica* on GenBank but were identified as *A. rostrata* and *A. anguilla* on BOLD, respectively.

A. japonica accessions from BOLD CCLF004-08, GBMIN95058-17, and GBMNA14388-19 were discarded from further analysis and tree building as they, despite coming from a validated database, came up as different species using BLAST against GenBank. Because of the ambiguities, these sequences were omitted from the analysis.

DISCUSSION

European eel trade

Despite international and regional regulations on trade, it was found that A. anguilla is still commonly available for purchase in Hong Kong retail outlets. On the basis of genetic tests, A. anguilla could be consistently detected across all retail outlets from convenience stores, middle class supermarkets, and high-end grocers. While many illicit wildlife trafficking channels are clandestine in nature and operate through specialized vendors, the unexpectedly high presence of A. anguilla in supermarkets suggests the ease and scale at which illegal wildlife products are able to move through mainstream distribution channels into what are perceived to be regulated and legitimate mass consumer markets. The relatively high percentage of A. anguilla in the species composition of eel products calls into question whether illicit products are entering the supply chain undetected. Furthermore, the lack of CITES-declared European eel imports into Hong Kong suggests that the eel found in supermarkets is likely to have been imported illegally, as we would expect higher instances of declared imports given the high proportion of European eel found in all the eel products surveyed.

Identification

All barcoding results were unambiguous and unanimous across the three different identification methods used. While cases of hybridization between Atlantic species (*A. anguilla* and *A. rostrata*) have been reported, they have been limited almost exclusively to populations in Iceland and occur at frequencies <20% (30, 31). Furthermore,

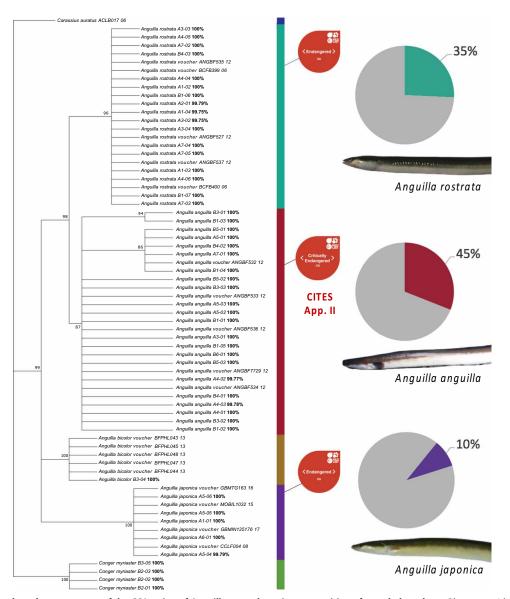


Fig. 1. Phylogenetic tree based on sequences of the COI region of Anguilla sp. and species composition of sampled products. Photos provided by Ryoshiro Wakiya, Florian M. Stein, and Erling Svensen (WWF).

CITES reports indicate a single exportation of Anguillidae specimens from Iceland in the past 10 years (https://trade.cites.org/). Considering evidence suggesting that hybridization of these two *Anguilla* species is exclusive to Iceland and that Iceland is not exporting large quantities of eel for commercial consumption, it is improbable that the effects of any hybridization events would impact the present study.

Emerging substitution and mislabeling

As *A. anguilla* glass eel recruitment from the ocean remains low, other species are being increasingly exploited (10, 32, 33). Already, large increases in the exploitation of the American eel (*A. rostrata*) and several tropical eel species (e.g., *Anguilla bicolor*, *Anguilla borneensis*, *Anguilla celebensis*, and *Anguilla marmorata*) have been documented and have led to increased national and international protections for these species. Regulations on the export of eel fry from Indonesia and the Philippines have been increasingly tightened in recent years

(32, 34–37). Exact trade levels from Southeast Asian countries remain unclear and vary significantly between information sources [e.g., Gollock et al. (35, 37)]. Nevertheless, Indonesia and the Philippines seem to be the major exporting countries for freshwater glass eels, while Thailand and Myanmar seem to export higher quantities of freshwater eels larger than glass eels (32, 37). Many of the Indo-Pacific Anguilla species are less well understood, making them particularly vulnerable to the combined pressures of habitat loss, environmental degradation, and possible exploitation for substitution in the international eel trade (35). Although present in our survey, A. japonica, A. bicolor, and A. rostrata are not currently listed in CITES Appendix II, and these species need to be closely monitored to see if their increasing presence in retail outlets is indicative of unsustainable thresholds.

As most products are only labeled eel, consumers are also unaware that they may be purchasing and consuming a critically endangered, CITES-protected species that may have been sourced through illegal means. Although species-specific labels are rare in supermarkets in Hong Kong, one raw *A. anguilla* eel fillet was mislabeled by the vendor as *A. japonica*. We noted that many of the European eel products surveyed indicated only "product of China" despite being of European origin, potentially misleading consumers.

Supply networks

The interconnected nature of all the retail outlets we surveyed suggests that there is only a handful of distributors responsible for all European eel products being sold to end consumers. Considering the fact that these retail groups operate supermarket chains with an international presence, this is an issue with regional if not global ramifications. A closer examination of the supply chain shows evidence that the same suppliers are used across the different brands, suggesting a consistent supplier ecosystem within each retail group. Suppliers were not specified for the majority of eel products; however, several of the high-end brands (A1, A2, A3, and A4) shared the same two suppliers. This provides further evidence that the same suppliers are often used across all brands under one retail group.

A deeper understanding of the centralized nature and key nodes of this distribution network could better inform disruption strategies for enforcement. Targeted enforcement aimed at key nodes within the network could disrupt supply chains across an entire retail group. Rather than identifying individual black market traders to halt retail at independent stores, targeting a few single suppliers could better fragment and disrupt the network as a whole.

Future prospects

These results provide an evidence-based understanding of the *A. anguilla* trade situation in Hong Kong, as well as valuable insight into the network distribution of such trafficking operations and effectiveness of current enforcement measures. Further investigation into the network structure of these supply chains would be invaluable for informed disruption strategies. We hope that the insight gained from this study will bring attention to the state of international illegal eel trade and help inform management and policy decisions going forward.

MATERIALS AND METHODS

Experimental design

Coverage of the surveyed retail outlets represented all major geographical regions and price tiers in Hong Kong. Sample collection was performed between November 2017 and February 2018. At every retail outlet surveyed, one of each eel product available was purchased and processed for identification, even when the same products were stocked across different stores. In general, Hong Kong supermarkets display considerable homogeneity in stocked products, particularly in convenience stores (vendors A6 and A7) for which the eel products purchased were all of the in-house private label brand.

Samples were composed of raw, frozen, previously cooked, and ready-to-eat (sushi) eel meat. DNA was extracted from 2 to 3 mm³ pieces of tissue based on the QIAGEN DNeasy kit (QIAGEN Inc.) manufacturer's protocol, along with a negative extraction control. All extractions were run on a 1.5% agarose gel and met visual quality standards, i.e., a bright, high-molecular weight band with little to no smearing. We then targeted a 655-base pair fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) for amplification

using polymerase chain reaction (PCR) using primers proven to be effective for fish species amplification and identification (38).

FishF1-5' TCA ACC AAC CAC AAA GAC ATT GGC AC 3' FishR1-5' TAG ACT TCT GGG TGG CCA AAG AAT CA 3'

Each 20-µl reaction contained 10 µl of Biotech Rabbit 2× Hotstart Lyophilized PCR Mastermix (Biotech Rabbit, Germany), 1 µM of each primer, and 2 µl of template DNA. PCR was carried out on a Veriti 96-Well Thermal Cycler (Applied Biosystems Inc.) with the following program: initial activation at 95°C for 2 min, followed by 35 cycles of denaturing at 95°C for 30 s, annealing at 48° for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. A no template control and a positive control containing DNA extracted from another fish species that had previously been amplified and sequenced successfully were also used.

PCR products were cycle sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems Inc.). Each cycle sequencing reaction consisted of 4.0 µl of BigDye, 3 µl of PCR product, 3 µl of ultrapure ddH₂O, and 1 µl of primer (3.2 pmol; COI FishF1 or COI FishR1). Sequencing was performed on an Applied Biosystems Genetic Analyzer 3130xl. Sequence chromatograms were assessed visually, and consensus sequences were generated in Geneious v10.0.7. Considering the closely related nature of the species in question (*A. anguilla, A. japonica*, and *A. rostrata*), the species identity of each sample was tested in three ways further detailed below: (i) comparison to entries within GenBank database using the BLAST algorithm and (ii) BOLD database using the BOLD ID engine, and (iii) phylogenetic tree building and species cluster delineations (see table S1) (39, 40).

First, consensus sequences for each *COI* barcoding region were compared to the GenBank sequence database for preliminary identification. To prevent potential misidentifications inherent in open source databases, the best matched sequence from GenBank was checked within BOLD, in which entries are more stringently validated. Several GenBank accessions did not match their BOLD IDs and so were not considered. We also compared sequences generated in this study directly within the BOLD database.

Last, a phylogenetic tree (Fig. 1) was constructed using reference sequences validated as mentioned above (BOLD and GenBank in agreement), and sequences from this study were mapped against it. The tree includes sequences generated in this study using *COI* primers and a subset validated and publicly available on the BOLD (www.barcodinglife.org) with accession numbers indicated. Sequences were aligned in MEGA7 (39) using the ClustalW algorithm, manually checked, and adjusted for a frameshift error. Model selection was also performed in MEGA7 with Tamura 3-parameter+I model yielding the lowest Bayesian Information Criterion score. The maximum-likelihood tree was constructed in MEGA7 using 1000 bootstrap replicates, a cutoff value of 85%, and support values indicated at each node. *Carassius auratus* was used as the outgroup.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/10/eaay0317/DC1
Table S1. Species ID table.

View/request a protocol for this paper from Bio-protocol.

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Prevalence of critically endangered European eel (*Anguilla anguilla*) in Hong Kong supermarkets

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